THE EFFECT OF STAMINODE REMOVAL ON FEMALE REPRODUCTIVE SUCCESS IN A WYOMING POPULATION OF THE ENDANGERED BLOWOUT PENSTEMON, *PENSTEMON HAYDENII* (SCROPHULARIACEAE)

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Abstract

We examined the effect of removing the staminode of the rare Blowout penstemon, *Penstemon haydenii*, on female reproductive success in a south-central Wyoming population. We found no significant difference in fruit set or in seeds per fruit between flowers with and without staminodes. The most frequent flower visitor, the megachilid bee, *Osmia brevis*, modified its behavior when visiting flowers without staminodes. In intact flowers, *O. brevis* collected pollen by straddling the staminode and rubbing its head and thorax across the anthers. In flowers without staminodes, *O. brevis*, appeared to compensate for the increased distance to the anthers from the corolla floor by simply stretching its legs to make contact with the anthers. The smaller, native generalist sweat bee (*Dialictus pruinosus*), which commonly collected pollen in intact flowers, appeared disoriented in flowers without staminodes, and frequently left without collecting pollen. Other taxa (e.g., bumblebees, *Bombus* spp., the masarid wasp *Pseudomasaris vespoides*), appeared unaffected and plied the flowers without incident. We speculate that the staminode of Blowout penstemon is intermittently vestigial: where *O. brevis*, bumblebees, and *P. vespoides* are common, the staminode is of little consequence; where *D. pruinosus* is the primary pollinator, the staminode is essential to sexual reproduction by the plant.

Key Words: bees, behavior, fecundity, *Penstemon*, pollinators, staminode.

Staminodes are stamens that have lost their essential male reproductive function: they no longer produce viable pollen and, in most instances, are antherless. Although not common, staminodes are found in about one-third of all Angiosperm families (Walker-Larsen and Harder 2000). They have evolved diverse shapes and functions (Ronse Decraene and Smets 2001), all seemingly related indirectly to sexual reproduction. For example, they may function as nectar guides (Delpino, cited in Straw 1956), act as a barrier to discourage flower entrance by nectar or pollen thieves (Straw 1956), provide a purchase to flower visitors (Pennell 1948), act as a lever mechanism to enhance pollination (Torchio 1974; Walker-Larsen and Harder 2001), or provide fodder to potential insect pollinators (Cane 1993). Or, they currently may serve no discernible function (Walker-Larsen and Harder 2001).

Staminodes are particularly common in the tribe Cheloneae (Scrophulariaceae), where they are thought to have accompanied the evolution of zygomorphic flowers (i.e., Endress 1999; Ronse Decraene and Smets 2001). In the genus *Penstemon*, staminodes are one of the defining characteristics: two pairs of fertile stamens, an upper and a lower, are positioned along the abaxial surface of the corolla, and the fifth sterile stamen,

the staminode, curves down to lie along the adaxial corolla "floor". The staminode is often densely pubescent, hence the common name, "beardtongue," for the genus. The staminode may be exerted, as in *P. eriantherus*, or extremely short and perpendicular to the corolla roof (e.g., *P. personatus*). These differences may be associated with the kind of primary pollinator that visits the flowers. Many of the hypotheses cited above to explain the evolution of staminodes have been presented with *Penstemon* in mind, but only recently have experimental studies begun to uncover the role of these structures (Walker-Larsen and Harder 2001; Dieringer and Cabrera R. 2002).

In this paper, we evaluate whether the staminode of Blowout penstemon, *Penstemon haydenii* S. Wats., plays a role in increasing female reproductive success of this rare species, and if the results differ with species of flower visitor (e.g., Dieringer and Cabrera R. 2002).

Penstemon haydenii is an early successional perennial herb of "blowouts" in sparsely vegetated, active sand dunes in western Nebraska and south-central Wyoming (Hardy et al. 1989; Stubbendieck et al. 1989; Heidel 2005). It has large (c. 25–30 mm), fragrant pale blue to lavender or pink flowers arranged in whorls (verticillasters) along the inflorescence. The staminode is included in the corolla, and is flattened

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at the distal end where it is densely to sparsely pubescent (or even glabrous) with yellow to golden hairs. Flowers are cross-pollinated and self-incompatible (Tepedino et al. 2006a, b). Nebraska and Wyoming populations are visited by a range of insects (Lawson et. al. 1989; Tepedino et al. 2006a, b; unpublished) but are pollinated primarily by native bees in the families Megachilidae, Halictidae and Apidae (Tepedino et al. 2006a, b). It has been listed as Endangered under the U. S. Endangered Species Act since 1987.

METHODS

We studied *P. haydenii* in the sand dunes of northwest Carbon Co., south-central Wyoming, USA, in June 2005. The Bear Mountain East site is situated on the south slope of Bear Mountain in a blowout depression in the Ferris Dunes (Stokes and Gaylord 1993) at the eastern end of the Ferris Mountains (2035–2270 m elevation). The *P. haydenii* ramets (c. 2000; Heidel 2005) grow among lemon scurf pea (*Psoralea lancelolata* Pursh.), chokecherry (*Prunus virginiana* L.), silverleaf phacelia (*Phacelia hastate* Dougl. Ex Lehm.), and painted milkvetch (*Astragalus ceramicus* Sheld. var. *filifolius* (A. Gray) Herm.). Average precipitation in the area is about 25 cm yr^{-1}, most falling in April and May. Consult Heidel (2005) for detailed site descriptions with maps and pictures.

Observations of flower phenology were made twice, once at the beginning of the blooming season (c. June 8) and again, about two weeks later when bloom was waning. Each time, ten fresh flowers (one on each of ten haphazardly selected plants) were observed for several successive days, from the beginning of anthesis to senescence. Anther dehiscence, stigma receptivity, and style position were noted.

To test for the role of the staminode in female reproductive success, we haphazardly selected 25 mature plants scattered throughout the population with an inflorescence with several mature, but unopened, buds in each of several flower whorls, and no open flowers. Inflorescences were bagged with white nylon tulle (1-mm^2 mesh) to prevent insect visitation before staminodes had been excised. To resist the frequent strong winds, the bag was anchored to the ground with metal wire. An additional unbagged inflorescence of similar age was chosen from a nearby plant as a control. As the buds opened on the bagged inflorescence, the staminode in two (rarely one) open flowers per node (up to eight flowers per inflorescence) was excised at the base with fine scissors while being held with forceps. Care was taken not to brush against the stamens and stigma during excision. For comparison, at least three flowers on each control treatment inflorescence were selected and marked with a paint pen. Fruits were collected in July 2005, air-dried in the lab and seeds were counted.

We compared fruits per flower and seeds per fruit between treatments using non-parametric statistical tests. Because not all whorls on each selected plant matured flowers, the distribution of treated flowers varied both across whorls and plants. We used the Chi square statistic to 1) compare fruit set among flowers of different whorls whose staminode had been removed, and 2) between the staminode removal treatment and controls. The Kruskal-Wallis test was used to compare seeds per fruit among different flower whorls whose staminode had been removed. Seeds per fruit between removal treatment and controls were compared using the Wilcoxon Rank Sum Test (also known as the Mann-Whitney test).

Behavior of abundant insect foragers on flowers with and without staminodes was observed and recorded soon after treatment during four 30-minute periods over two days.

RESULTS

The developmental tempo of flower maturation accelerated with time. At the beginning of the blooming season, flowers remained open for four to seven days. The outer pair of anthers typically dehisced on the second day and the inner pair the next day. The downward hook in the style, normally taken to signify stigma receptivity (Straw 1956; but see Tepedino et al. 1999) occurred by the second day (i.e., at the same time the outer pair of anthers were dehiscing). Flowers of later-blooming plants had flowers that opened and senesced in one to two days. Both pairs of anthers dehisced and the style hooked on the first day. Both maturation sequences are unusual in *Penstemon* because the style usually does not bend below the anthers until after the anthers dehisce (see also Tepedino et al. 1999).

Plants in the treatment groups, particularly the open-pollinated treatment, were heavily browsed by cattle and wild ungulates. Nine of the inflorescences used for staminode removals and almost all of the control inflorescences on adjacent plants were cropped, forcing us to compare open-pollinated control plants from a concurrent breeding system study (Tepedino et al. 2006b) with the treatment flowers.

Before comparing results from the staminode removal experiments with controls, we compared fruits per flower and seeds per fruit among the four flower whorls (Table 1). There was no difference among whorls in fruits per flower (χ^2 = 7.44, df = 3, P > 0.05) or in seeds per fruit (Kruskal-Wallis Test, df = 3, P > 0.40) for the staminode removal treatment; we therefore com-

	Frts	NoFrts	N	Seeds/Frt	SD
Staminode	60	26	16	12.5	2.9
Removed					
Open Pollinated	21	7	10	15.9	6.2
Whorl 1	24	3	14	11.0	5.3
Whorl 2	14	11	13	12.0	6.5
Whorl 3	14	7	11	14.5	5.5
Whorl 4	8	5	7	12.9	3.0

bined the data for comparison with the open-pollinated treatment.

We found no difference in fruit set between the staminode removal flowers and the open-pollinated treatment (Table 1; $\chi^2 = 0.28$, df = 1, P > 0.50). Nor was there a difference in seeds per fruit between treatments (Wilcoxon Rank Sum Test, P > 0.30). Apparently, female reproductive success was little influenced by the absence of the staminode.

The most abundant visitors to the flowers of Blowout penstemon were bees of the genera *Osmia* (especially *O. brevis*), *Dialictus* spp., *Bombus* spp. and the masarid wasp, *Pseudomasaris vespoides,* a specialized *Penstemon* visitor (Cooper 1952). All were seen visiting flowers with and without staminodes. In some cases, the floral visitors modified their behavior on the flowers without staminodes. For example, on intact flowers, *O. brevis* females would land on the staminode and rub their head and thorax back and forth on the anthers to collect pollen, concurrently contacting any stigma that had reached the hooked stage). When visiting flowers without a staminode, *O. brevis* landed on the floor of the corolla and stretched upwards to reach the anthers. We saw no indication, either in observations, or when modest pressure was applied to the staminode, of declination of the stamens or style as occurs when the staminode functions as a lever (Torchio 1974).

Dialictus females seemed disoriented by staminode removal. In staminodeless flowers, they appeared unable to gain a purchase on the corolla floor and soon left. Normally, they spent much time on the flowers, crawling around inside the corolla, perhaps to collect nectar; frequently resting on the staminode or walking over the stamens and anthers in an inverted position to become dusted with pollen on the ventral abdomen. In general, *Dialictus* females spent more time on the flowers than did other bees.

(Dieringer and Cabrera R. 2002 also found that small bees spent longer times on the flowers than did large bees.)

Two visitors did not modify their behavior. *Pseudomasaris vespoides* females exhibited the same pollen collecting technique in flowers with and without staminodes. Like *O. brevis*, they collected pollen by scraping their heads against the anthers. However, because of their size, female *P. vespoides* had no difficulty in reaching the anthers. Similarly, *Bombus* spp. did not show any obvious behavioral changes when visiting flowers with staminodes removed.

DISCUSSION

The production of an elaborate staminode, as in Blowout penstemon, would seem to entail a non-trivial cost of energy and nutrients. One expects such an expenditure to be compensated for by a benefit, such as an increase in reproductive success. Recently, two groups have followed early studies by Clements and Long (1923) by removing the staminodes of five species and examining the effects on female and male reproductive success (Dieringer and Cabrera R. 2001, 2002; Walker-Larsen and Harder 2001). Their results suggest that the function of the staminode in *Penstemon* species is diverse, and that even well-developed, costly staminodes may not have an obvious function. Results of staminode removals by Clements and Long (1923) were also species-specific; our findings support that interpretation.

Walker-Larsen and Harder (2001) examined four species, two bird-pollinated (*P. centranthifolius*, *P. rostriflorus*) and two bee-pollinated (*P. ellipticus*, *P. palmeri*), with very different results. The bird-pollinated species exhibited no effect of staminode removal, either on pollen deposition on the stigma, or pollen removal from the anthers. Results for the bee-pollinated species were more complicated: pollen deposition on the stigma decreased significantly for flowers without staminodes but the mechanism of action was different for *P. ellipticus* and *P. palmeri*. Pollen removal was significantly greater from anthers in intact *P. palmeri* flowers than from flowers without staminodes. However, greater pollen removal rates need not translate to greater male fitness: that depends on whether pollen is deposited on a receptive stigma or is removed from the system either by grooming or by being using as larval food (Thomson and Thomson 1992; Kobayashi et al. 1999). In *P. ellipticus*, pollen removal was unaffected by staminode removal.

The work of Dieringer and Cabrera R. (2001, 2002) also shows an effect of staminode removal on female reproductive success of a bee-pollinated species, *P. digitalis*. Initially (2001), they showed that flowers with staminodes removed

produced significantly fewer seeds per fruit than intact flowers. Subsequently, they showed that staminode removal affected pollen deposition on the stigma but not pollen removal from the anthers (Dieringer and Cabrera R. 2002) and that the size of flower-visiting bees was important: both large and small (but not intermediate-sized) bees deposited fewer pollen grains in staminodeless flowers. Thus, for all three bee-pollinated species studied thus far, pollen deposition per visit is reduced by staminode removal. In contrast, male reproductive success may have been lower in only one of the three species.

Time constraints prevented us from estimating pollen deposition and removal. Instead, we used a simple bioassay to compare fruit set and seed production between flowers with staminodes and flowers whose staminodes were removed (Dieringer and Cabrera R. 2001). In contrast to Walker-Larsen and Harder (2001) and Dieringer and Cabrera R. (2001, 2002), we found no effect of staminode removal on female reproductive success of this bee- and wasp-pollinated beardtongue.

Our result would appear to be another example of species-specific differences. Evidently, it is not simply that the staminode is superfluous in bird-pollinated species and functional in bee-pollinated species as found by Walker-Larsen and Harder (2001). *Penstemon haydenii* may be a bee-pollinated species whose staminode function is dependent upon the pollinators that service it. The behavior of larger bees and wasps (*Osmia, Bombus, Pseudomasaris*) on flowers without staminodes seemed unlikely to depress either pollen delivery or removal. Conversely, the much smaller *Dialictus* bees completely changed their behavior on flowers without staminodes such that pollination was unlikely. Similarly, Dieringer and Cabrera R. (2002) showed that pollen delivery was pollinator-size dependent; intermediate sized bees (but not small or large bees) were unaffected in pollen delivery by absence of a staminode. These results are not novel: Clements and Long (1923) long ago reported that some foraging bees are unperturbed by many types of experimental flower modifications.

We suspect that the staminode of Blowout penstemon may influence reproductive success only in some years and at some sites, when smaller visitors like *Dialictus pruinosus* predominate. Whether this occurs frequently enough to support the continued presence of the staminode in this species over the long term is an open question.

Literature Cited

Cane, J. H. 1993. Reproductive role of sterile pollen in *Saurauia* (Actinidiaceae), a cryptically dieocious neotropical tree. Biotropica 25:493–495.

Clements, F. E. and F. L. Long. 1923. Experimental pollination: An outline of the ecology of flowers and insects. Carnegie Institute of Washington Publication No. 336, Washington D.C.

Cooper, K. W. 1952. Records and flower preferences of masarid wasps. II. Polytrophy or oligotrophy in *Pseudomasaris*? American Midland Naturalist 48:103–110.

Dieringer, G. and L. Cabrera R. 2001. Pollination ecology of bristle staminodia in *Penstemon digitalis* (Scrophulariaceae) and *Jacaranda acutifolia* (Bignoniaceae). Biotam 12:31–36.

———— and ————. 2002. The interaction between pollinator size and the bristle staminode of *Penstemon digitalis* (Scrophulariaceae). American Journal of Botany 89:991–997.

Endress, P. K. 1999. Symmetry in flowers: diversity and evolution. International Journal of Plant Sciences 160(Supplement): S3–S23.

Hardy, J. P., R. R. Weedon, and W. R. Bowlin. 1989. Blowout Penstemon: Description and present situation. Pp. 227–231 *in* T. B. Bragg and J. Stubbendieck (eds.), Proceedings 11th North American Prairie Conference. Lincoln, NE.

Heidel, B. 2005. *Penstemon haydenii* (blowout Penstemon) – 2004 monitoring establishment report, Carbon County, Wyoming. U.S. Department of the Interior, Bureau of Land Management, Rawlins, WY.

Kobayashi, S., K. Inoue, and M. Kato. 1999. Mechanism of selection favoring a wide tubular corolla in *Campanula punctata*. Evolution 53: 752–757.

Lawson, H. R., V. J. Tepedino, and T. L. Griswold. 1989. Pollen collectors and other insect visitors to *Penstemon haydenii* S. Wats. Pp. 233–235 *in* T. B. Bragg and J. Stubbendieck (eds.), Proceedings 11th North American Prairie Conference. Lincoln, NE.

Pennell, F. W. 1948. The taxonomic significance of an understanding of floral evolution. Brittonia 6: 301–308.

Ronse Decraene, L. P. and E. F. Smets. 2001. Staminodes: Their morphological and evolutionary significance. Botanical Review 67:351–402.

Stokes, S. and D. R. Gaylord. 1993. Optical dating of Holocene dune sands in the Ferris dune field. Quaternary Research 39:274–281.

Straw, R. M. 1956. Adaptive morphology of the *Penstemon* flower. Phytomorphology, July:112–118.

Stubbendieck, J., T. R. Flessner, and R. R. Weedon. 1989. Blowouts in the Nebraska sandhills: The habitat of *Penstemon haydenii*. Pp. 223–225 *in* T. B. Bragg and J. Stubbendieck (eds.),

Proceedings 11th North American Prairie Conference. Lincoln, NE.

TEPEDINO, V. J., S. D. SIPES, AND T. L. GRISWOLD. 1999. The reproductive biology and effective pollinators of the endangered beardtongue *Penstemon penlandii* (Scrophulariaceae). Plant Systematics and Evolution 219:39–54.

———, W. R. BOWLIN, AND T. L. GRISWOLD. 2006a. The pollination biology of the endangered Blowout Penstemon (*Penstemon haydenii* S. Wats.: Scrophulariaceae) in Nebraska. Journal of the Torrey Botanical Society 133:548–559.

———, T. R. TOLER, B. A. BRADLEY, J. L. HAWK, AND T. L. GRISWOLD. 2006b. Pollination biology of a disjunct population of the endangered sandhills endemic *Penstemon haydenii* S. Wats. (Scrophulariaceae) in Wyoming, USA. Plant Ecology: Online First. DOI 10.1007/S11258-006-9248-7.

THOMSON, J. D. AND B. A. THOMSON. 1992. Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. Pp. 1–24 *in* R. Wyatt (ed.), Ecology and evolution of plant reproduction. Chapman & Hall, New York, NY.

TORCHIO, P. F. 1974. Mechanisms involved in the pollination of *Penstemon* visited by the masarid wasp, *Pseudomasaris vespoides* (Cresson). Pan-Pacific Entomologist 50:226–234.

WALKER-LARSEN, J. AND L. D. HARDER. 2000. The evolution of staminodes in angiosperms: patterns of stamen reduction, loss, and functional reinvention. American Journal of Botany 87: 1367–1384.

——— AND ———. 2001. Vestigal organs as opportunities for functional innovation: the example of the *Penstemon* staminode. Evolution 55:477–487.